29

The repellency portion of this study will be conducted according to the current type protocol "Testing Non-Standard Insect Repellents and Repellent Formulations in Volunteers". This protocol was approved by the Surgeon General's Human Subjects Research Review Board in October 1981. The test 5 method was subsequently adopted by the American Society for Testing and Materials (ASTM) in 1989.

Generally, a formulation comprising an equal amount of sunscreen and arthropod repellent will be applied to the flexor region of the forearms of human volunteers. The repellent formulations will be applied at random to the flexor region of the forearms of four volunteers. There are 8 groups to be tested including 2 control groups. At the start of the test, a 4×5×18 cm plastic cage containing 15 mosquitoes will be bound to forearm with Velcro® tape, and a slide will be 15 withdrawn to expose the repellent treated skin. The number of mosquitoes biting in the test cage will be recorded at the end of 90 seconds. New mosquitoes will be used in each test and the cages will be removed after 90 seconds. This test procedure will be repeated every two hours for 10 hours. Thus, six 20 tests of each species of mosquitoes will be conducted on each formulation after application on the skin. The above procedure will be repeated four times. The temperature and relative humidity in the test room will be recorded on the test days. The 5% end will be employed in each test. Since there will be 25 15 mosquitoes per cage and 6 test periods (0, 2, 4, 6, 8, and 10 hours), this means that a test can be terminated whenever 5% of the 15×6 mosquitoes have bitten. Thus a test may be discontinued after 5 bites have been received on the repellent treated area on the forearm.

Percent repellency will be determined from the total number of bites on the control and repellent treated volunteers by converting to percentages of the total for the control and subtracting from 100.

% Repellency = 
$$100 - \left(\frac{\text{# of bites on treatment}}{\text{# of bites on control}} \times 100\right)$$

Sun protection factors (SPF) of the combination sunscreen and arthropod repellent formulations will be determined in accordance with the Federal monograph of proposed rules for sunscreen testing published in the Federal Register, Vol. 43, No. 166, 25 Aug. 1978, which is herein incorporated by reference. The standard against which new sunscreen formulations are measured is an 8% Homosalate formulation (SPF 4).

Minimal erythemal dose (MED) is defined as the time interval or dosage of UVR sufficient to produce a minimal, perceptible erythema on untreated skin. Prior to the testing phase, the MED of each subject is determined by a progressive sequence of timed UVR exposures, each of which is graduated incrementally by 25% over that of the previous site. Twenty-four hours after irradiation, the sites are evaluated from erythema according to the following scoring system.

0 Negative, no visible reaction

- +/- Minimal, perceptible erythema
- 1+ Defined erythema
- 2+ Moderate erythema
- 3+ Severe erythema

A sufficient number of 5×10 cm test site areas are outlined with a surgical marking pen on the subject's back between the

30

scapulae and the beltline, lateral to the midline. These areas are designated for the test material(s) or standard, with an adjacent site designated for a concurrent MED determination (unprotected control). A 0.1 ml or 0.1 g portion of test material(s) or standard is applied to the appropriate  $5\times10$  cm test site and spread evenly over the site using a fingercot. This delivers a film of  $2 \text{ mg/cm}^2$ .

At least 15 minutes after the product application, the test site is divided into subsites which are used for a defined serial UVR exposure.

Exposure times are selected for each subsite in treated areas based upon the previously determined MED of the untreated skin and the anticipated SPF of the test material(s) or standard.

Sun protection factor is defined as the ratio of the amount of energy required to produce an MED on protected skin (treated with test material(s) or standard) to the amount of energy needed to produce an MED on untreated skin and is calculated as follows:

$$SPF = \frac{\text{Minimal Erythema Dose in sun-protected skin}}{\text{Minimal Erythema Dose in non-sunscreen-protected skin}}$$

To the extent necessary to understand or complete the disclosure of the present invention, all publications, patents, and patent applications mentioned herein are expressly incorporated by reference therein to the same extent as though each were individually so incorporated.

Having thus described exemplary embodiments of the present invention, it should be noted by those skilled in the art that the within disclosures are exemplary only and that various other alternatives, adaptations, and modifications may be made within the scope of the present invention. Accordingly, the present invention is not limited to the specific embodiments as illustrated herein, but is only limited by the following claims.

We claim:

- 1. A compound which is 2-allylsufanyl-3-methyl-pyrazine.
- 2. A composition comprising at least one compound of according to claim 1.
- 3. The composition of claim 2, and further comprising a pharmaceutically or cosmetically acceptable carrier.
- **4**. The composition of claim **2**, wherein the composition is a lotion, a cream, a foam, an aerosol, a face paint, a stick, a soap, a sunscreen product, or a cosmetic.
- 5. A method of repelling an arthropod from a surface of a substrate, an area, or a mammal which comprises administering to, placing or immobilizing on or in, integrating on or in the surface, the area, or the mammal an effective amount of 2-allylsufanyl-3-methyl-pyrazine.
- 6. The method of claim 5, wherein the substrate is a fabric, an article of clothing, a bed net, a curtain, a paper, a wall paper, a window screen, a ground cloth, a tent, a towelette, or a protective over garment.
- 7. The method of claim 5, wherein the compound is formulated into a lotion, a cream, a foam, an aerosol, a face paint, or a stick
- **8**. The method of claim **5**, wherein the compound is integrated into a soap, a sunscreen product, or a cosmetic.

\* \* \* \* \*